Interface
focus

REVIEW

Interconnected porous hydroxyapatite ceramics for bone tissue engineering

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Several porous calcium hydroxyapatite (HA) ceramics have been used clinically as bone substitutes, but most of them possessed few interpore connections, resulting in pathological fracture probably due to poor bone formation within the substitute. We recently developed a fully interconnected porous HA ceramic (IP-CHA) by adopting the 'foam-gel' technique. The IP-CHA had a three-dimensional structure with spherical pores of uniform size (average 150 μm, porosity 75%), which were interconnected by window-like holes (average diameter 40 μm), and also demonstrated adequate compression strength (10–12 MPa). In animal experiments, the IP-CHA showed superior osteoconduction, with the majority of pores filled with newly formed bone. The interconnected porous structure facilitates bone tissue engineering by allowing the introduction of mesenchymal cells, osteotropic agents such as bone morphogenetic protein or vasculature into the pores. Clinically, we have applied the IP-CHA to treat various bony defects in orthopaedic surgery, and radiographic examinations demonstrated that grafted IP-CHA gained radiopacity more quickly than the synthetic HA in clinical use previously. We review the accumulated data on bone tissue engineering using the novel scaffold and on clinical application in the orthopaedic field.

Keywords: bone; ceramics; hydroxyapatite; tissue engineering; mesenchymal cell

1. INTRODUCTION

When bone grafts are required for bony defects in orthopaedic surgery, autogenous bone grafting has been the gold standard because of its obvious advantages in osteogenic potential, mechanical properties and the lack of adverse immunological response. On the other hand, autogenous bone grafting has some limitations, such as the requirement of additional surgery for harvesting, the availability of sufficient grafts in size and shape and the risk of donor-site morbidity (Banwart *et al.* 1995; Arrington *et al.* 1996), which may include long-lasting pain, fracture, nerve damage and infection. Although allogeneic bone is widely used in the USA, its use is quite limited in Japan, accounting for as little as 3 per cent of procedures (Prolo & Rodrigo 1985), presumably owing to religious difficulties with using tissue from other people or corpses, as well as the lack of a well-organized tissue bank system. In addition, allograft carries the risk of transmission of occult disease, or a host immune response, which can sometimes result in complete resorption of the grafts. Therefore, many kinds of biomaterials have been developed as bone substitutes,

such as hydroxyapatite (HA), alumina, zirconia, bioglass, polymers, metal, and organic or inorganic bone substitutes (Sartoris *et al.* 1986; Bucholz *et al.* 1987; Fujibayashi *et al.* 2003; Nishikawa & Ohgushi 2004).

HA ceramics have been used extensively as a substitute in bone grafts (Holmes *et al.* 1987; Bucholz *et al.* 1989), because the crystalline phase of natural bone is similar to HA. Since the 1980s, blocks and granules of porous calcium HA ceramics (CHA) have been used in orthopaedic, dental or craniofacial surgery (Uchida *et al.* 1990; Yoshikawa & Uchida 1999; Matsumine *et al.* 2004). However, there are few reports which indicate that the pores of implanted CHA are totally filled with newly formed host bone (Nakasa *et al.* 2005), probably owing to the closed structures of these CHA with few interpore connections (Ayers *et al.* 1998).

Therefore, the development of porous CHA with interpore connections of adequate diameter as well as adequate strength has long been expected as an ideal bone substitute (Roy *et al.* 2003; Simon *et al.* 2003, 2007, 2008). We recently developed a fully interconnected porous HA ceramic (IP-CHA; porosity 75%, average pore size 150 μm and average interconnections 40 μm) by adopting a 'foam-gel' technique, crosslinking polymerization that gelatinizes through the foam-like slurry in a moment (Tamai *et al.* 2002). The interconnected porous structure facilitates bone
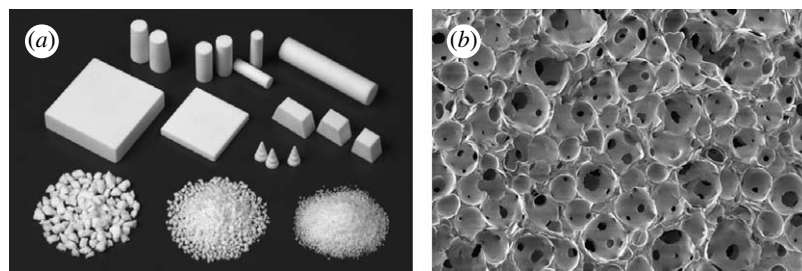
Figure 1. Interconnected porous HA ceramics (IP-CHA). (*a*) Macroscopic images of IP-CHA. The materials were manufactured by Covalent Materials Corporation. (*b*) SEM image of the microstructures of IP-CHA. Spherical pores (100–200 µm in diameter) are divided by thin walls and interconnected by interpores (10–80 µm in diameter).

tissue engineering by allowing the introduction of mesenchymal cells, osteotropic agents or vasculature into the pores. In this review, we report a new bone tissue engineering system using IP-CHA, a preliminary clinical result in patients treated with IP-CHA and a new clinical trial using a prefabricated IP-CHA in orthopaedic surgery.

2. CONVENTIONAL HYDROXYAPATITE CERAMICS IN JAPAN

The crystalline phase of natural bone is basically HA, and HA ceramics have been used extensively as a substitute in bone grafts. The ceramics are available as dense or porous types and the shape types are granular or block-like. Different pore sizes, porosities and strengths are available. Here, we describe four types of conventional HA (the first generation) that were used clinically.

— *BONEFIL* (*Mitsubishi Materials Corporation*). The types of ceramics are porous blocks and porous granules, and are most often used in orthopaedics. The sintering temperature is 900°C and the compression strength is 15 MPa/2 to 3 MPa. The pore shape is spongiose and the pore size is 200–300 µm. The degree of porosity is 60–70 per cent.

— *BONETITE* (*Mitsubishi Materials Corporation*). The types of ceramics are porous blocks and dense granules, and are most often used in dental surgery. The sintering temperature is 1200°C. The pore shape is spongiose and the pore size is 200 µm. The degree of porosity is 70 per cent.

— *BONECERAM* (*Sumitomo Osaka Cement Co. Ltd*). Porous block and porous granular types of the ceramics are available as BONECERAM-P. The sintering temperature is 1150°C. The compression strength is 44.1–68.6 MPa and the bending strength is 12.7–19.6 MPa. The pore shape is spherical and the pore size is 50–300 µm. The degree of porosity is 35–48 per cent. Dense block types of the ceramics having a high mechanical strength are available as BONECERAM-K. The sintering temperature is 1150°C. The bending strength is over 58.8 MPa.

— *APACERAM* (*PENTAX Corporation*). The types of ceramics are both dense and porous. The porous ceramic has a degree of porosity of 15–60 per cent. The sintering temperature is 1200°C. The compression and bending strengths vary from 16 to 250 MPa and 8 to 47 MPa, respectively. The better mechanical properties are associated with a decrease in the degree of porosity. The pore shape is spherical. The pore structure is an interconnected bimodal pore configuration consisting of a combination of 300 µm macropores and 2 µm micropores. The dense HA has a degree of porosity of less than 0.8 per cent. The sintering temperature is 1050°C. The compression and bending strengths are 750 and 210 MPa, respectively. Clinical applications began in 1985, with approximately 5000 clinical uses of APACERAM ceramics (custom-designed porous type plate) in cranioplasty since then. The numbers of clinical cases involving spinal surgery and ENT surgery with ear ossicle substitutes are 70 000 and 20 000, respectively.

All four of these manufactured HA ceramics are without effective interpore connections and essentially non-resorbable.

3. INTERCONNECTED POROUS HYDROXYAPATITE CERAMICS

The conventional method used to manufacture synthetic porous HA ceramics is by sintering a HA slurry mixed with organic polymer beads (Uchida *et al.* 1984). The polymer beads melt and vaporize during the sintering process, eventually leaving pores in the ceramic material. However, the pores resulting from this method are irregular in size and shape and not fully interconnected with one another. Together with Covalent Materials Corporation, MMT Co. Ltd and National Institute for Materials Science, Biomaterials Center, we developed an IP-CHA (porosity 75%, average pore size 150 µm and average interpore connections 40 µm) by adopting the foam-gel technique (figure 1; Tamai *et al.* 2002). This approach involves a crosslinking polymerization step that gelatinizes the foam-like CHA slurry in a rapid manner, thus promoting the formation of an interconnected porous structure. Briefly, the new method is as follows. (i) Slurry preparation: the slurry was prepared by mixing HA (60 wt%) with a crosslinking substrate (polyethyleneimine, 40 wt%). (ii) Foaming and gelatinization: the slurry was mixed with a foaming agent (polyoxyethylene lauryl ether, 1 wt%) and stirred until the mixture had a foamy appearance. Pore size was controlled by regulating the stirring time.
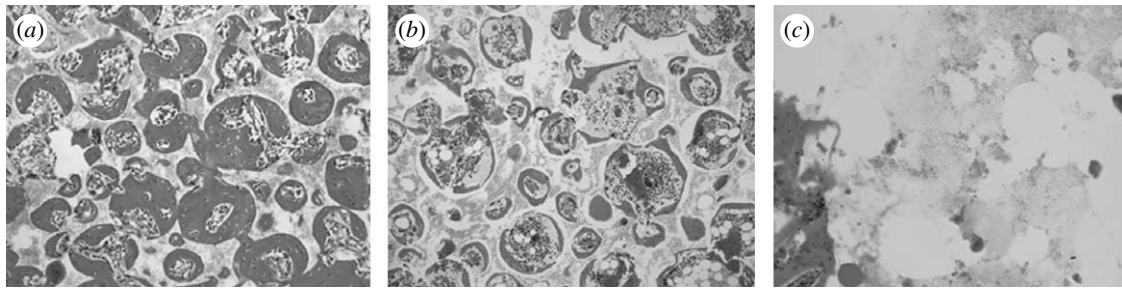
Figure 2. New bone formation within the IP-CHA in rabbit femoral condyle (HE staining×100). (*a*) Most of the pores were filled with newly formed bone at 2 weeks and (*b*) bone marrow formation was detected at 6 weeks. (*c*) In contrast, bone formation was not observed in the control group (conventional HA without interpore connections).
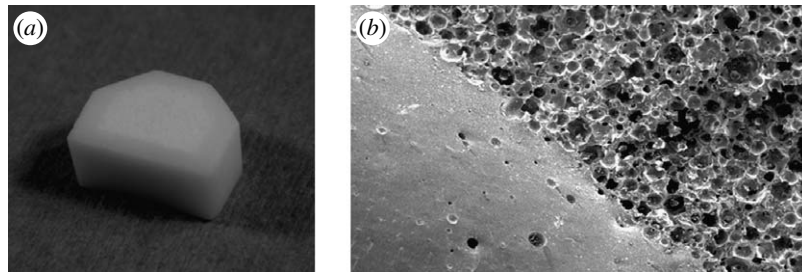

Figure 3. (*a*) Macroscopic and (*b*) microscopic (SEM) images of the solid/interconnected porous HA composite. Mechanical strength of the solid and the porous part is 550–570 and 10–12 MPa, respectively.

(iii) Gelatinization: to gelatinize the foamed slurry, another water-soluble crosslinking agent (polyfunctional epoxy compound) was added and the mixture was cast by pouring into a mould. The porous structure stabilized in less than 30 min. The foamy HA gel was removed from the mould, dried and sintered at 1200°C.

Scanning electron microscopy (SEM) analysis revealed that most of the IP-CHA pores were spherical, similar in size, approximately 100–200 μm in diameter, and showed uniform connections with one another. The wall surface of IP-CHA was very smooth and HA particles were aligned closely to one another and bound tightly.

The majority of the interpore connections ranged from 10 to 80 μm in diameter, with a maximum peak of approximately 40 μm, which would theoretically allow cell migration or tissue invasion from pore to pore (Steinkamp *et al.* 1976). Interpore connections larger than 10 μm accounted for as much as 91 per cent of the total porosity in IP-CHA. The calculated available porosity, the proportional volume of pores in the material that were connected by interpore connections larger than 10 μm in diameter, was 73.4 per cent (total porosity)×0.91=67.1 per cent. The compression strength was 12 MPa, while the compression strength of cancellous bone is 1–12 MPa (Martin *et al.* 1993).

4. OSTEOCONDUCTION *IN VIVO*

Macroporosity is known to influence the biological performance of calcium phosphate *in vivo*. Holmes *et al.* (1988) reported that pores of approximately 100 μm in diameter could provide a framework for bone growth into the pore, which then becomes vascularized easily. Most of the pores of IP-CHA are large enough to show such criteria and, more importantly, the pores are fully interconnected and more likely to allow bone ingrowth.

Cylindrical blocks (6 mm in diameter) of IP-CHA were implanted into rabbit femoral condyle, and the bone ingrowth was histologically analysed (Tamai *et al.* 2002; Myoui *et al.* 2004; Yoshikawa & Myoui 2005). Within six weeks after implantation of IP-CHA, mature bone ingrowth was seen in most of the pores throughout the block. In the pores, bone, bone marrow formation through interpore connections with osteoblastic rimming and vessels were all observed (figure 2). We also examined the sequential change in the compression strength of IP-CHA implanted in rabbit femoral condyle. The initial compression strength of IP-CHA was approximately 10–12 MPa. The implanted IP-CHA steadily increased its compression strength with time until nine weeks after implantation, finally reaching a value of approximately 30 MPa (Tamai *et al.* 2002).

Recently, in order to reinforce its initial mechanical strength, we have developed a novel composite with the solid form of HA. Figure 3 shows the macroscopic and microscopic images of the solid/interconnected porous HA composite (Kaito *et al.* 2006). The mechanical strength of the solid part is 550–570 MPa, thus the solid part may correspond to cortical bone, and the porous part to cancellous bone. We constructed an implant and used a canine lumbar interbody fusion model to evaluate bone conduction of the implant and its efficacy for bony fusion. Six months after the surgery, the implant exhibited almost the same efficacy for bony fusion as iliac bone grafts. Moreover, pores of the porous part of the implant were completely filled with newly formed bone and bone marrow cells (Kaito *et al.* 2006).

5. CLINICAL APPLICATION IN ORTHOPAEDIC SURGERY

HA is a useful material to fill bone defects in treating benign bone tumours because of its biocompatibility,
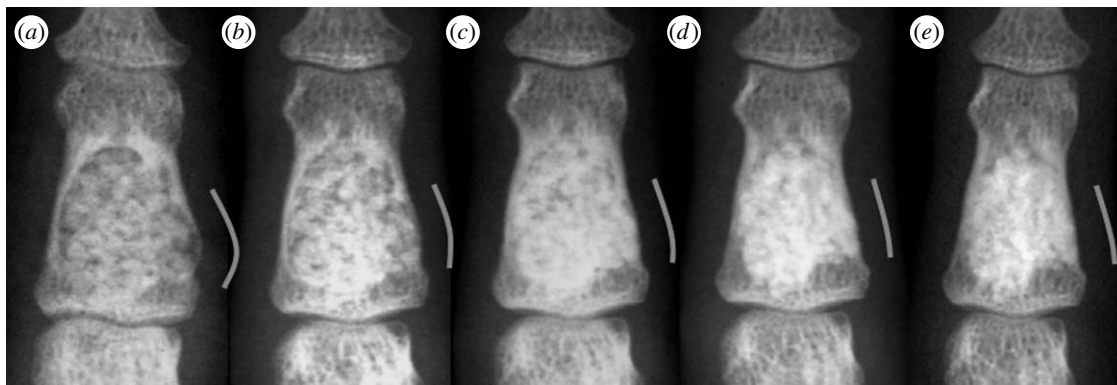
Figure 4. Clinical application of IP-CHA in the treatment of patients with bone tumours. An enchondroma of mid-phalanx, 28-year-old male. As radiodensity increased, the affected bone was remodelled, and the expansive deformity was self-corrected. (*a*) Just after surgery, (*b*) 3 months after surgery, (*c*) 6 months after surgery, (*d*) 12 months after surgery and (*e*) 27 months after surgery.
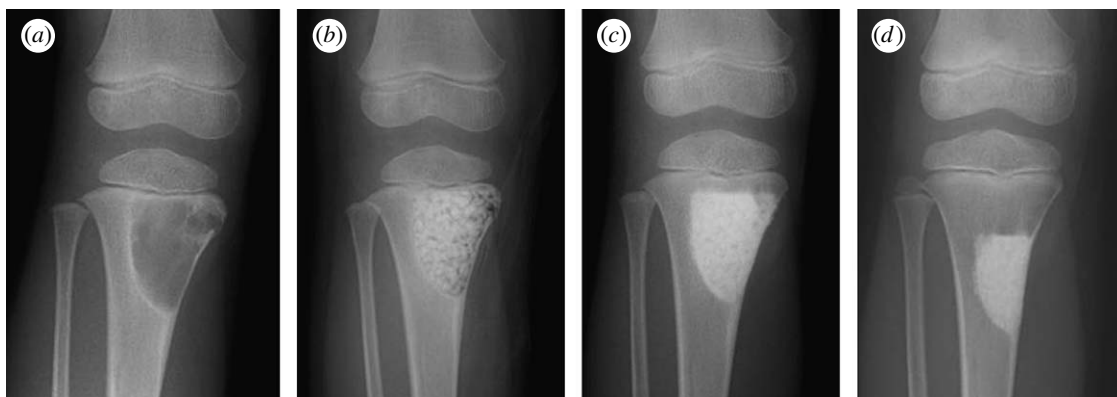

Figure 5. Clinical application of IP-CHA in the treatment of patients with bone tumours. A simple bone cyst of proximal tibia, 5-year-old boy. (*a*) Before surgery, (*b*) just after surgery, (*c*) 6 months after surgery and (*d*) 36 months after surgery.

osteoconduction and convenience, and it eliminates the need for additional surgery for harvesting autograft, as we reported previously (Uchida *et al.* 1990; Yoshikawa & Uchida 1999; Matsumine *et al.* 2004). However, as a late complication, pathological fractures of the implanted sites have been reported (Yoshikawa & Uchida 1999; Matsumine *et al.* 2004). This is probably due to poor bone ingrowth in the material as a result of poor incorporation of the material into the host bone. We applied IP-CHA as a bone substitute for the treatment of 59 patients with benign bone tumours at the Osaka University Hospital and its affiliated hospitals. The average age of patients was 32 years (range 5–75 years). The tumours were located in the upper extremities in 25 patients, lower extremities in 27 and pelvis in 7. The mean follow-up period was 46 months (range 32–60 months). After adequate removal of the tumours, IP-CHA blocks and/or granules of 2–5 mm in diameter were used to fill the bony defects. We also used IP-CHA to fill 12 cystic lesions with rheumatoid arthritis (Shi *et al.* 2006). None of the patients showed any signs of inflammatory reaction, rejection, infection or abnormal results in blood tests. Neither pathological fracture nor deformity was observed at the implanted site based on radiographic examinations during the follow-up period. The radiographic examinations were periodically carried out and revealed that the radiolucent line between the implanted IP-CHA and host bone tended to decrease with time after surgery and eventually disappeared (figure 4). The radiographic density at the implanted site increased with time and the IP-CHA granules appeared to fuse with one another, eventually forming a dense radiopaque shadow. Interestingly, longitudinal bone growth was not disturbed even when IP-CHA was implanted in close proximity to the growth plate of children (figure 5). Gadolinium-enhanced MRI showed a ring enhancement at the periphery of the implant (data not shown) and the area with enhancement advanced towards the centre of the implant, indicating that bone regeneration with blood supply might occur within the IP-CHA.

The IP-CHA can be prefabricated into specific sizes and shapes to match bone defects. We did this to make an implant, which was, in advance, planned and reconstructed with a computer-aided design/manufacturing (CAD/CAM) system. A three-dimensional image was reconstructed with the CT data of the estimated bony defect, and the IP-CHA was fabricated by a three-dimensional milling machine (Roland DG, MDX-20; figure 6). We have used the prefabricated IP-CHA for various bony defects in orthopaedic surgery, and obtained a satisfactory clinical outcome.
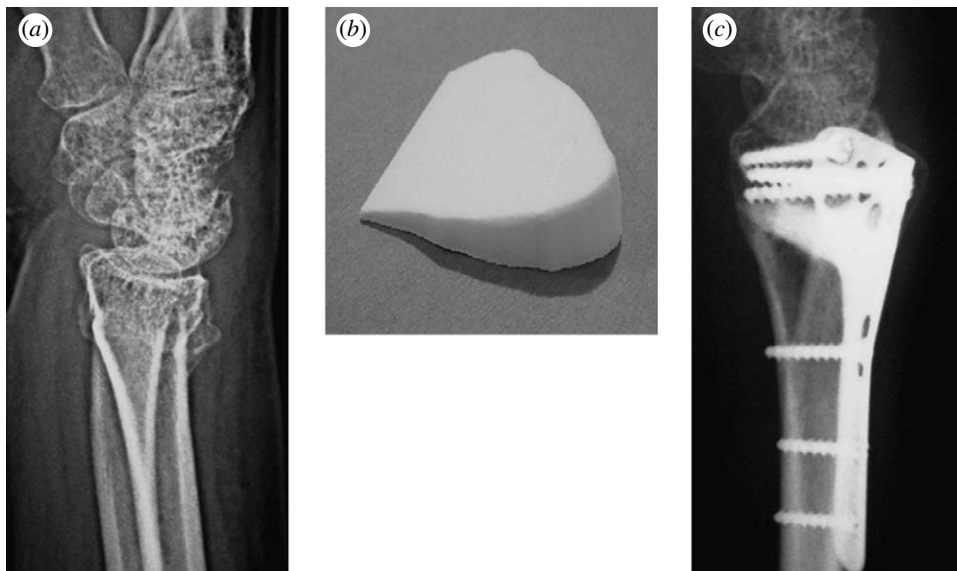
Figure 6. Correction osteotomy for malunited fracture of the distal radius, 48-year-old woman. (*a*) Preoperative X-ray image of the affected radius, six months after the fracture of the distal radius. (*b*) Prefabricated IP-CHA. A three-dimensional image was reconstructed with the CT data of the estimated bony defect after correction, and the IP-CHA was fabricated by a three-dimensional milling machine (Roland DG, MDX-20). (*c*) Post-operative X-ray image, a year after surgery. The malalignment was satisfactorily corrected by the surgery using the prefabricated IP-CHA and a metal plate.
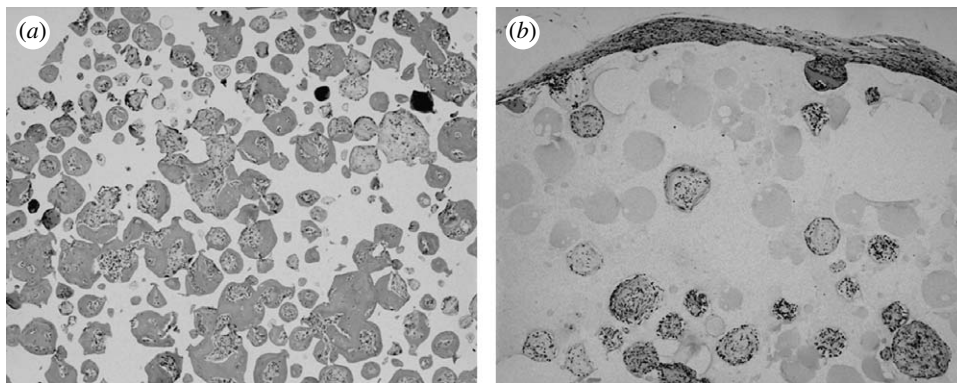

Figure 7. Bone tissue engineering by mesenchymal stem cells (HE staining×40). (*a*) At eight weeks after implantation, extensive bone volume was detected not only in the surface pore areas but also in the centre pore areas of IP-CHA. (*b*) In the control group (conventional HA without interpore connections), little bone formation was observed even in the surface pore areas.

6. BONE TISSUE ENGINEERING BY MESENCHYMAL STEM CELLS

The IP-CHA can be used as a scaffold for cell-based bone tissue engineering. We tested the efficacy of IP-CHA using a rat subcutaneous model by Ohgushi & Caplan (1999). Bone marrow cells were collected from the femur of rat and were cultivated in minimal essential medium supplemented with 15 per cent foetal bovine serum. IP-CHA discs ($R=5$ mm, $h=2$ mm) were soaked in the cell suspension overnight and further cultured in the same medium with β-glycerophosphate, ascorbic acid and dexamethasone for 14 days. The discs were then implanted into the subcutaneous tissue of rats and harvested for two to eight weeks after implantation. All the implants showed bone formation inside the pore areas as evidenced by decalcified histological sections and microcomputed tomography images (Nishikawa *et al.* 2004, 2005). At eight weeks after implantation, extensive bone volume was detected not only in the surface pore areas, but also in the centre pore areas of the implants (figure 7). The combination of IP-CHA and mesenchymal cells could be used as an excellent bone graft substitute because of its mechanical properties and capability of bone formation.

Recently, we have started a clinical trial with the combination of IP-CHA and autologous mesenchymal cells for bone tissue repair, and already treated 10 patients.

A precise clinical evaluation is necessary, but we believe that bone tissue engineering by IP-CHA offers new approaches to treatment for patients requiring skeletal reconstruction.

7. BONE TISSUE ENGINEERING BY BONE MORPHOGENETIC PROTEIN

Bone morphogenetic proteins (BMPs) are biologically active molecules capable of inducing new bone formation, and show potential for clinical use in bone defect repair

Figure 8. Bone healing by IP-CHA combined with BMP. (*a*) No implant group at eight weeks after surgery. No bone formation was detected. (*b*) IP-CHA group alone at eight weeks after implantation. Radiolucent lines were clearly visible between IP-CHA and host bone. (*c*) rhBMP-2 (5 µg)/IP-CHA group at eight weeks after implantation. Bony unions were observed at the junction sites and the radiodensity of IP-CHA increased.

(Wozney & Rosen 1998; Nakase & Yoshikawa 2006). However, an ideal system for delivering BMPs that can potentiate their bone-inducing ability and provide initial mechanical strength and scaffold for bone ingrowth has not yet been developed. We have analysed the efficacy of IP-CHA as a delivery system for recombinant human BMP-2 (rhBMP-2). We combined two biomaterials to construct a carrier/scaffold system for rhBMP-2: IP-CHA and a synthetic biodegradable polymer, poly-D,L-lactic acid–polyethyleneglycol block co-polymer (PLA-PEG; Miyamoto *et al.* 1993; Saito *et al.* 2001). A rabbit radius model was used to evaluate the bone-regenerating activity of the rhBMP-2/PLA-PEG/IP-CHA composite. All bone defects in groups treated with 5 µg of rhBMP-2 were completely fixed with sufficient strength at eight weeks after implantation (Kaito *et al.* 2005; figure 8). Using this carrier scaffold system, we reduced the amount of rhBMP-2 necessary for such results to approximately one-tenth of the amount needed in previous studies. Enhancement of bone formation is probably due to the superior osteoconduction ability of IP-CHA and the optimal drug delivery system provided by PLA-PEG. The PLA-PEG/IP-CHA composite is an excellent carrier/scaffold delivery system for rhBMP-2, and strongly encourages the clinical effects of rhBMP-2 in bone tissue regeneration.

8. BONE TISSUE ENGINEERING BY VASCULAR PREFABRICATION

Vascular network invasion into porous implants is another important aspect of using such materials as bone substitutes for large bone defects or in the construction of tissue-engineered bone, because cells cannot survive farther than a few hundred micrometres from a nutrient supply. The rate of new bone ingrowth into the porous material depends on vascular invasion from the surface of the implant, which is not fast enough in large implants to transport nutrients to cells transplanted in pores of the implant. Therefore, we

examined whether prefabrication of IP-CHA with a vascular bundle enhances vascular network invasion into the pores via interpore connections (Akita *et al.* 2004; Myoui *et al.* 2004; Yoshikawa & Myoui 2005). When an IP-CHA cylindrical block was prefabricated with rat superficial inferior epigastric vessels, vascular invasion in the pores increased in both number and size, when compared with the control, resulting in more abundant fibrous connective tissue formation. Our findings suggest that inserting a vascular bundle into such interconnecting porous implants at the site of implantation supports vascular network invasion, which may eventually enhance bone ingrowth in the implants. Nakasa *et al.* (2005) reported that prefabrication of vascularized bone graft using a combination of fibroblast growth factor-2 and vascular bundle implantation into IP-CHA led to a satisfactory result in the reconstruction of bony defects.

9. APPLICATION FOR CARTILAGE REPAIR AND TENDON ATTACHMENT

We have developed a new technology for articular cartilage repair, consisting of a triple composite of rhBMP-2, PLA-PEG and IP-CHA, to induce the regeneration of both subchondral bone and articular cartilage (Tamai *et al.* 2005). Full-thickness cartilage defects in the rabbit were filled with the rhBMP-2 (20 µg)/PLA-PEG/IP-CHA composite. At six weeks, subchondral defects were completely repaired by subchondral bone and articular cartilage covering the bone. The regenerated cartilage manifested a hyaline-like appearance, with a columnar organization of chondrocytes and a mature matrix. The novel cell-free technology, the triple composite of rhBMP-2, PLA-PEG and IP-CHA, could mark a new development in the field of articular cartilage repair. Our new strategy for articular cartilage repair seems to be unique for the following three reasons: (i) we used autogenous mesenchymal cells efficiently recruited from bone

marrow by strongly activating the regeneration process of the subchondral bone defect, (ii) continuous BMP stimuli seemed to promote both the vigorous regeneration of subchondral bone and the following chondrocytic differentiation and cartilaginous matrix production at the surface resulting in hyaline-like cartilage regeneration in as little as three weeks, and (iii) the regenerated cartilage exhibited almost perfect lateral integration with the surrounding host cartilage, probably because the whole regeneration process in this system was *in situ* and efficient, unlike an *ex vivo* chondrocyte culture system. Ito *et al.* (2008) reported that an osteochondral plug using cultured chondrocytes and cylindrical IP-CHA plugs was successful in treating osteochondral defects in a rabbit model. Ohmae *et al.* have also tried to enhance tendon attachment to bone using IP-CHA with bone marrow stromal cells in a rabbit model, and obtained a satisfactory result (Omae *et al.* 2006, 2007).

10. CONCLUSIONS

The foam-gel technique is an innovative method that generates a three-dimensional fully interconnected porous structure in synthetic HA ceramics. The interconnected porous structure encourages bone ingrowth into the material and eventually leads to good incorporation of the material into the host bone. Our study indicated that IP-CHA exhibited excellent bone ingrowth in an animal model and favourable performance in clinical use. We believe that IP-CHA is an excellent bone substitute for filling bone defects and should be considered as an alternative to autogenous bone. In addition, IP-CHA seems likely to serve as a good scaffold for cell-based or cytokine-based tissue-engineered bone. In fact, we have been successful in bone tissue engineering using rhBMP-2, mesenchymal cells or vasculature in animals. The synthetic scaffold can be prefabricated into specific sizes and shapes to match bone defects, and even into a composite with the solid form of HA in order to reinforce its initial mechanical strength. IP-CHA is now commercially available in Japan, and we have applied IP-CHA as a bone substitute for the treatment of more than 80 patients with benign bone tumours or rheumatoid arthritis, and obtained some favourable clinical results. Recently, we have started a clinical trial with the combination of IP-CHA and autologous mesenchymal cells for bone tissue repair. Additional studies with larger animals including dogs or monkeys and precise clinical evaluation are necessary, but we believe that bone tissue engineering by IP-CHA offers new approaches to the treatment of patients requiring skeletal reconstruction.

REFERENCES

Akita, S., Tamai, N., Myoui, A., Nishikawa, M., Kaito, T., Takaoka, K. & Yoshikawa, H. 2004 Capillary vessel network integration by inserting a vascular pedicle enhances bone formation in tissue-engineered bone using interconnected porous hydroxyapatite ceramics. *Tissue Eng.* **10**, 789–795. (doi:10.1089/1076327041348338)

Arrington, E. D., Smith, W. J., Chambers, H. G., Bucknell, A. L. & Davino, N. A. 1996 Complications of iliac crest bone graft harvesting. *Clin. Orthop.* **329**, 300–309. (doi:10.1097/00003086-199608000-00037)

Ayers, R. A., Simske, S. J., Nunes, C. R. & Wolford, L. M. 1998 Long-term bone ingrowth and residual micro hardness of porous block hydroxyapatite implants in humans. *J. Oral Maxillofac. Surg.* **56**, 1297–1301. (doi:10.1016/S0278-2391(98)90613-9)

Banwart, J. C., Asher, M. A. & Hassanein, R. S. 1995 Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. *Spine* **20**, 1055–1060. (doi:10.1097/00007632-199505000-00012)

Bucholz, R. W., Carlton, A. & Holmes, R. E. 1987 Hydroxyapatite and tricalcium phosphate bone graft substitute. *Orthop. Clin. North Am.* **18**, 323–334.

Bucholz, R. W., Carlton, A. & Holmes, R. 1989 Interporous hydroxyapatite as a bone graft substitute in tibial plateau fractures. *Clin. Orthop.* **240**, 53–62.

Fujibayashi, S., Kim, H. M., Neo, M., Uchida, M., Kokubo, T. & Nakamura, T. 2003 Repair of segmental long bone defect in rabbit femur using bioactive titanium cylindrical mesh cage. *Biomaterials* **24**, 3445–3451. (doi:10.1016/S0142-9612(03)00221-7)

Holmes, R. E., Bucholz, R. W. & Mooney, V. 1987 Porous hydroxyapatite as a bone graft substitute in diaphyseal defects: a histometric study. *J. Orthop. Res.* **5**, 114–121. (doi:10.1002/jor.1100050114)

Holmes, R. E., Wardrop, R. W. & Wolford, L. M. 1988 Hydroxylapatite as a bone graft substitute in orthognathic surgery: histologic and histometric findings. *J. Oral Maxillofac. Surg.* **46**, 661–671. (doi:10.1016/0278-2391(88)90109-7)

Ito, Y., Adachi, N., Nakamae, A., Yanada, S. & Ochi, M. 2008 Transplantation of tissue-engineered osteochondral plug using cultured chondrocytes and interconnected porous calcium hydroxyapatite ceramic cylindrical plugs to treat osteochondral defects in a rabbit model. *Artif. Organs* **32**, 36–44.

Kaito, T., Myoui, A., Takaoka, K., Saito, N., Nishikawa, M., Tamai, N., Ohgushi, H. & Yoshikawa, H. 2005 Potentiation of the activity of bone morphogenetic protein-2 in bone regeneration by a PLA-PEG/hydroxyapatite composite. *Biomaterials* **26**, 73–79. (doi:10.1016/j.biomaterials.2004.02.010)

Kaito, T., Mukai, Y., Nishikawa, M., Ando, W., Yoshikawa, H. & Myoui, A. 2006 Dual hydroxyapatite composite with porous and solid parts: experimental study using canine lumbar interbody fusion model. *J. Biomed. Mater. Res. B* **78**, 378–384. (doi:10.1002/jbm.b.30498)

Martin, R. B., Chapman, M. W., Sharkey, N. A., Zissimos, S. L., Bay, B. & Shors, E. C. 1993 Bone ingrowth and mechanical properties of coralline hydroxyapatite 1 yr after implantation. *Biomaterials* **14**, 341–348. (doi:10.1016/0142-9612(93)90052-4)

Matsumine, A., Myoui, A., Kusuzaki, K., Araki, N., Seto, M., Yoshikawa, H. & Uchida, A. 2004 Calcium hydroxyapatite

ceramic implants in bone tumor surgery. A long-term follow-up study. *J. Bone Joint Surg. Br.* **86**, 719–725. (doi:10.1302/0301-620X.86B5.14242)

Miyamoto, S., Takaoka, K., Okada, T., Yoshikawa, H., Hashimoto, J., Suzuki, S. & Ono, K. 1993 Polylactic acid-polyethylene glycol block copolymer: a new biodegradable synthetic carrier for bone morphogenetic protein. *Clin. Orthop.* **294**, 333–343.

Myoui, A., Tamai, N., Nishikawa, M., Araki, N., Nakase, T., Akita, S. & Yoshikawa, H. 2004 Three-dimensionally engineered hydroxyapatite ceramics with interconnected pores as a bone substitute and tissue engineering scaffold. In *Biomaterials in orthopedics* (eds M. J. Yaszemski, D. J. Trantolo, K. U. Lewandrowski, V. Hasirci, D. E. Altobelli & D. L. Wise), pp. 287–300. New York, NY: Marcel Dekker.

Nakasa, T., Ishida, O., Sunagawa, T., Nakamae, A., Yasunaga, Y., Agung, M. & Ochi, M. 2005 Prefabrication of vascularized bone graft using a combination of fibroblast growth factor-2 and vascular bundle implantation into a novel interconnected porous calcium hydroxyapatite ceramic. *J. Biomed. Mater. Res.* **75A**, 350–355. (doi:10.1002/jbm.a.30435)

Nakase, T. & Yoshikawa, H. 2006 Potential roles of bone morphogenetic proteins (BMPs) in skeletal repair and regeneration. *J. Bone Mineral Metab.* **24**, 425–433. (doi:10.1007/s00774-006-0718-8)

Nishikawa, M. & Ohgushi, H. 2004 Calcium phosphate ceramics in Japan. In *Biomaterials in orthopedics* (eds M. J. Yaszemski, D. J. Trantolo, K. U. Lewandrowski, V. Hasirci, D. E. Altobelli & D. L. Wise), pp. 425–436. New York, NY: Marcel Dekker.

Nishikawa, M., Myoui, A., Ohgushi, H., Ikeuchi, M., Tamai, N. & Yoshikawa, H. 2004 Bone tissue engineering using novel interconnected porous hydroxyapatite ceramics combined with marrow mesenchymal cells: quantitative and three-dimensional image analysis. *Cell Transplant.* **13**, 367–376. (doi:10.3727/000000004783983819)

Nishikawa, M., Ohgushi, H., Tamai, N., Osuga, K., Uemura, M., Yoshikawa, H. & Myoui, A. 2005 The effect of simulated microgravity by three-dimensional clinostat on bone tissue engineering. *Cell Transplant.* **14**, 829–835. (doi:10.3727/000000005783982477)

Ohgushi, H. & Caplan, A. I. 1999 Stem cell technology and bioceramics: from cell to gene engineering. *J. Biomed. Mater. Res.* **48A**, 913–927. (doi:10.1002/(SICI)1097-4636(1999)48:6<913::AID-JBM22>3.0.CO;2-0)

Omae, H., Mochizuki, Y., Yokoya, S., Adachi, N. & Ochi, M. 2006 Effects of interconnecting porous structure of hydroxyapatite ceramics on interface between grafted tendon and ceramics. *J. Biomed. Mater. Res. A* **79**, 329–337. (doi:10.1002/jbm.a.30797)

Omae, H., Mochizuki, Y., Yokoya, S., Adachi, N. & Ochi, M. 2007 Augmentation of tendon attachment to porous ceramics by bone marrow stromal cells in a rabbit model. *Int. Orthop.* **31**, 353–358. (doi:10.1007/s00264-006-0194-8)

Prolo, D. J. & Rodrigo, J. J. 1985 Contemporary bone graft physiology and surgery. *Clin. Orthop.* **200**, 322–342. (doi:10.1097/00003086-198511000-00036)

Roy, T. D., Simon, J. L., Ricci, J. L., Rekow, E. D., Thompson, V. P. & Parsons, J. R. 2003 Performance of degradable composite bone repair products made via three-dimensional fabrication techniques. *J. Biomed. Mater. Res. A* **66**, 283–291. (doi:10.1002/jbm.a.10582)

Saito, N., Okada, T., Horiuchi, H., Murakami, N., Takahashi, J., Nawata, M., Ota, H., Miyamoto, S., Nozaki, K. &

Takaoka, K. 2001 Biodegradable poly-D,L-lactic acid-polyethylene glycol block copolymers as a BMP delivery system for inducing bone. *J. Bone Joint Surg.* **83A**, S92–S98.

Sartoris, D. J., Gershuni, D. H., Akeson, W. H., Holmes, R. E. & Resnick, D. 1986 Coralline hydroxyapatite bone graft substitutes: preliminary report of radiographic evaluation. *Radiology* **159**, 133–137.

Shi, K., Hayashida, K., Hashimoto, J., Sugamoto, K., Kawai, H. & Yoshikawa, H. 2006 Hydroxyapatite augmentation for bone atrophy in total ankle replacement in rheumatoid arthritis. *J. Foot Ankle Surg.* **45**, 316–321. (doi:10.1053/j.jfas.2006.06.001)

Simon, J. L., Roy, T. D., Parsons, J. R., Rekow, E. D., Thompson, V. P., Kemnitzer, J. & Ricci, J. L. 2003 Engineered cellular response to scaffold architecture in a rabbit trephine defect. *J. Biomed. Mater. Res. A* **66**, 275–282. (doi:10.1002/jbm.a.10569)

Simon, J. L., Michna, S., Lewis, J. A., Rekow, E. D., Thompson, V. P., Smay, J. E., Yampolsky, A., Parsons, J. R. & Ricci, J. L. 2007 *In vivo* bone response to 3D periodic hydroxyapatite scaffolds assembled by direct ink writing. *J. Biomed. Mater. Res. A* **83**, 747–758. (doi:10.1002/jbm.a.31329)

Simon, J. L., Rekow, E. D., Thompson, V. P., Beam, H., Ricci, J. L. & Parsons, J. R. 2008 MicroCT analysis of hydroxyapatite bone repair scaffolds created via three-dimensional printing for evaluating the effects of scaffold architecture on bone ingrowth. *J. Biomed. Mater. Res. A* **85**, 371–377.

Steinkamp, J. A., Hansen, K. M. & Crissman, H. A. 1976 Flow microfluorometric and light-scatter measurement of nuclear and cytoplasmic size in mammalian cells. *J. Histochem. Cytochem.* **24**, 292–297.

Tamai, N., Myoui, A., Tomita, T., Nakase, T., Tanaka, J., Ochi, T. & Yoshikawa, H. 2002 Novel hydroxyapatite ceramics with an interconnective porous structure exhibit superior osteoconduction *in vivo*. *J. Biomed. Mater. Res.* **59A**, 110–117. (doi:10.1002/jbm.1222)

Tamai, N., Myoui, A., Hirao, M., Kaito, T., Ochi, T., Tanaka, J., Takaoka, K. & Yoshikawa, H. 2005 A new bio-technology for articular cartilage repair: subchondral implantation of a composite of interconnected porous hydroxyapatite, synthetic polymer (PLA/PEG), and bone morphogenetic protein-2 (rhBMP-2). *Osteoarthr. Cartil.* **13**, 405–417. (doi:10.1016/j.joca.2004.12.014)

Uchida, A., Nade, S. M., McCartney, E. R. & Ching, W. 1984 The use of ceramics for bone replacement. A comparative study of three different porous ceramics. *J. Bone Joint Surg. Br.* **66**, 269–275.

Uchida, A., Araki, N., Shinto, Y., Yoshikawa, H., Kurisaki, E. & Ono, K. 1990 The use of calcium hydroxyapatite ceramic in bone tumour surgery. *J. Bone Joint Surg. Br.* **72**, 298–302.

Wozney, J. M. & Rosen, V. 1998 Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. *Clin. Orthop.* **346**, 26–37. (doi:10.1097/00003086-199801000-00006)

Yoshikawa, H. & Myoui, A. 2005 Bone tissue engineering with porous hydroxyapatite ceramics. *J. Artif. Organs* **8**, 131–136. (doi:10.1007/s10047-005-0292-1)

Yoshikawa, H. & Uchida, A. 1999 Clinical application of calcium hydroxyapatite ceramic in bone tumor surgery. In *Biomaterials and bioengineering handbook* (ed. D. L. Wise), pp. 433–455. New York, NY: Marcel Dekker.